

Graduate School of Pharmaceutical Sciences¹, Kumamoto University, Kumamoto; Faculty of Pharmacy and Pharmaceutical Sciences², Fukuyama University, Fukuyama, Japan; Faculty of Pharmacy³, Al-Minya University, El-Minia, Egypt; Faculty of Pharmaceutical Sciences⁴, Sojo University, Kumamoto, Japan

Enhancement of dissolution and bioavailability of flurbiprofen by low molecular weight chitosans

M. ANRAKU^{1,2}, M. ARAHIRA¹, F. M. MADY^{1,3}, K. A. KHALED³, K. YAMASAKI⁴, H. SEO⁴, T. IMAI¹, M. OTAGIRI^{1,4}

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Professor Masaki Otagiri, Ph.D., Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan
otagirim@gpo.kumamoto-u.ac.jp

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The dissolution behavior and absorption of flurbiprofen (FP) following oral administration from three types of chitosans (LM chitosans), with different molecular weights and degree of acetylation, have been studied in comparison with those of the drug alone. The solubility of FP increased with concentrations of LM chitosan, especially in the case of C-III, with the highest degree of deacetylation degree among the three chitosans. This indicates that amino groups of LM chitosan play an important role in its interaction with FP. Moreover, spectroscopic studies, including NMR data, indicate that the binding involves interactions between the carboxyl group of FP and the amino group of the chitosans. The dissolution rates of FP for a C-III kneaded mixture were enhanced with increasing amounts of C-III. The oral absorption of FP from a C-III kneaded mixture was improved to a significant extent, compared to FP alone. These results suggest that FP from LM chitosan kneaded mixture increases the dissolution rate and improves the bioavailability of the drug by the formation of a water-soluble complex.

1. Introduction

Natural polymers such as polysaccharides and proteins are a subject of interest in the pharmaceutical field owing to their good biocompatibility and biodegradability (Abdelbary and Tadros, 2008; Imai et al. 1989; Miyazaki et al. 2006). Among the class of polysaccharides, chitosan, the deacetylated product produced by the alkaline treatment of chitin, is thought to be one of the most useful natural polymers, and has been studied as a carrier material for new delivery systems (Felt et al. 1998; Ilium 1998; Mutalik et al. 2008; Paul and Sharma 2000; Tomida et al. 2010). Chitosans with different degrees of acetylation (DA, 10–50%) were synthesized by the partial acetylation of deacetylated chitosan using different ratios of acetic anhydride. In previous studies, it was reported that the rate of degradation of acetylated chitosans can be controlled by appropriate adjustment of the DA value: degradation increases with increasing DA value of the acetylated chitosans (Lim et al. 2008). Chitosan is believed to affect lipid concentrations by binding via its positively charged amino groups to negatively charged substrates, such as fats and lipids in the gastrointestinal tract, thus preventing their absorption (Bokura and Kobayashi 2003; Sumiyoshi and Kimura 2006). It was previously reported that low molecular weight chitosans (LM chitosans, M.W. 950–3800), produced by the hydrolysis of chitosan, create a hydrophilic surface on drug particles by virtue of the dispersion of the drug into the

chitosan, thereby enhancing the dissolution rate of a poorly water-soluble drug (Shiraishi et al. 1990). Moreover, it would be predicted that chitosan, a basic polysaccharide, would interact with acidic drugs to a greater extent than with basic drugs. However, interactions between chitosan and acidic drugs have not been studied extensively, because of the low solubility of chitosan. It has been suggested that the interaction of LM chitosan with indomethacin as an acidic model drug, can be useful for improving the dissolution and absorption rates of indomethacin (Imai et al. 1991).

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs worldwide, and are widely prescribed for patients with rheumatic diseases (Heyneman et al. 2000). Flurbiprofen (FP), 2-(2-fluoro-4-biphenyl) propionic acid, exerts analgesic, anti-inflammatory, and anti-pyretic activity via the inhibition of the enzymatic activity of cyclooxygenase, leading to the suppression of prostaglandin synthesis (Hardman et al. 2001).

The present study was mainly concerned with interactions between LM chitosan and FP as an acidic model drug. In addition, some pharmaceutical properties of a kneaded mixture such as dissolution rate and absorption behavior were examined and compared with pure FP powder.

2. Investigations, results and discussion

2.1. Interaction in aqueous solutions

Figure 1 shows equilibrium phase solubility diagrams obtained for FP with LM chitosans in water at 25 °C. The solubility of FP

Abbreviations: FP, flurbiprofen; DA, different degrees of acetylation; LM, low molecular weight; DSC, differential scanning calorimetry; C_{max}, maximum plasma concentration; AUC, the plasma concentration-time curve.

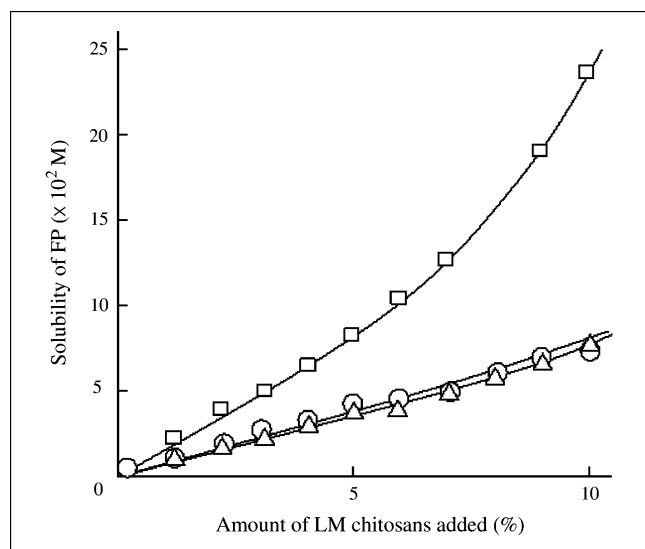


Fig. 1: Phase solubility diagrams of FP-chitosan systems in water at 25 °C. (○) FP-C-I system; (△) FP-C-II system; (□) FP-C-III system. Each data point represents the average of 3 experiments

in the presence of C-I, C-II and C-III increased with increasing amounts of LM chitosan and the solubility curve can be classified as Type A (Higuchi and Connors 1965). The solubility of FP increased in the order of C-III > C-II > C-I. Since chitosan is a basic polysaccharide, the increasing solubility of FP, which is an acidic drug, might have been due to the buffer capacity of chitosan (the pH of the aqueous solution: 7.6–8.7). However, C-I and C-II have almost the same degree of deacetylation in solution (Table 1). The only difference between C-I and C-II is the molecular weight. Therefore, the increasing solubility of FP can be attributed to a difference in the interaction mode of FP with LM chitosan. This result indicates that the amino groups in chitosan play an important role in the interaction with FP. In fact, the fluorescence of FP was quenched upon the addition of LM chitosans (20–25% decrease, compared with the original FP fluorescence). Moreover, D-glucosamine also significantly quenched the fluorescence of FP (approximately 80% quenching) but N-acetyl-D-glucosamine did not. This strongly supports the importance of the amino group in LM chitosan in the binding process.

To further investigate the interactions between FP with LM chitosans, ^{13}C NMR spectra were obtained. Figure 2 shows the effects of C-III on the ^{13}C -NMR spectra of FP. The values in the figure are the difference in chemical shifts of FP in the absence and presence of C-III. Negative signs indicate an upfield shift. The ^{13}C signals of the biphenyl ring of FP showed no appreciable change as the result of binding to C-III. On the other hand, the other carbons which do not reside in the ring, were significantly affected by the complexation with LM chitosan. The propionic acid portion of the molecule (C-13, 15) and C4 showed a significant downfield shift, suggesting an electrostatic interaction with the amino group of glucosamine residues. It appears that

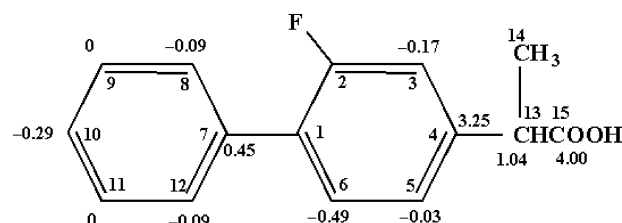


Fig. 2: Effect of C-III on the ^{13}C -chemical shifts of FP. The values are the difference in chemical shifts of FP in the absence and presence of C-III. Negative signs indicate an upfield shift

the free rotation of the biphenyl ring decreases in solution as the result of the formation of a complex between FP and chitosan. From the above observations, it can be concluded that the amino groups of LM chitosan and the carboxyl groups of FP play an important role in complex formation.

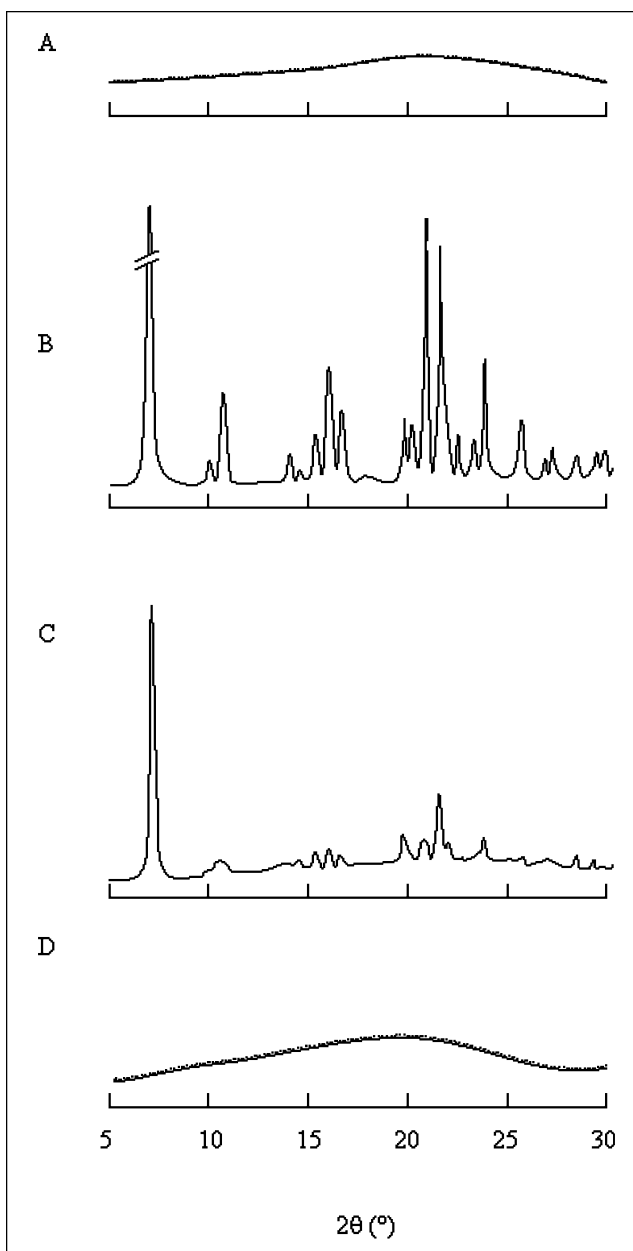


Fig. 3: Powder X-ray diffraction patterns of FP-C-III systems. (A) C-III alone; (B) FP alone; (C) physical mixture of FP and C-III (1:3); (D) FP-C-III complex (1:3)

Table 1: Physicochemical properties of LM chitosans

	C- I	C- II	C- III
Molecular weight (M.W.)	3800	1700	950
Deacetylation degree (%)	65	68	83
Aqueous solubility (g/100 ml)	>50	>50	>50
Intrinsic viscosity (dl/g)	0.14	0.08	0.06
Decomposition temperature (°C)	225	222	220

2.2. Interaction in solid state

X-ray diffractometry, and differential scanning calorimetry (DSC) were employed to examine the interaction of FP with LM chitosans in the solid state and to compare it with that of the corresponding physical mixture. Figure 3 shows powder X-ray diffraction patterns of the FP-C-III complex, compared with FP and C-III itself, as a typical example. The diffraction pattern of C-III showed a halo pattern over the 2θ range of $5\text{--}30^\circ$, owing to the fact that it is a freeze dried preparation. The diffraction peaks of the FP-C-III complex were broader than those in the physical mixture. In addition, the diffraction peaks that were observed from 20° to 24° at 2θ in the physical mixture disappeared as the result of complex formation. These data suggest that the FP-C-III complex causes a decrease in crystallinity and microcrystal size, causing a change in the crystal lattice and microcrystal shape (Alexander 1969), and that FP powder exists as separate crystals in the LM chitosan dispersion. The decrease in crystallinity and crystal size of the FP-C-III complex may enhance the dissolution rate owing to an improvement in wettability (Shiraishi et al. 1990).

Figure 4 shows the DSC thermograms of the FP-C-III complex, FP and C-III itself. C-III exhibited no endothermic peak owing to its amorphous form. In the case of FP, an endothermic peak due to the melting of FP was observed at around 120°C . In sharp contrast, the thermogram of the complex showed only a broad endothermic peak at around 113°C , which can be attributed to the elimination of water from the complex after drying. Furthermore, the disappearance of the sharp endothermic peak of FP in the FP-C-III system may be due to the formation of a complex between the carboxylic groups of FP and the amino groups of LM chitosan.

2.3. Dissolution behavior

Figure 5 shows the dissolution profiles of FP and kneaded mixtures containing LM chitosan at three different ratios in water at 25°C . The kneaded mixtures exhibited a significantly increased rate of dissolution compared to that of FP alone. It was previously reported that the rate of dissolution of kneaded mixtures was faster than that of physical mixtures with decreasing contact angle on drug particles (Shiraishi et al. 1990). The rates of dissolution for FP from kneaded mixtures were enhanced with increasing amounts of C-III. Thus the pharmaceutical characteristics of the kneaded mixture of FP are more acceptable than those of FP. Hence, the 1:2 kneaded mixtures which showed

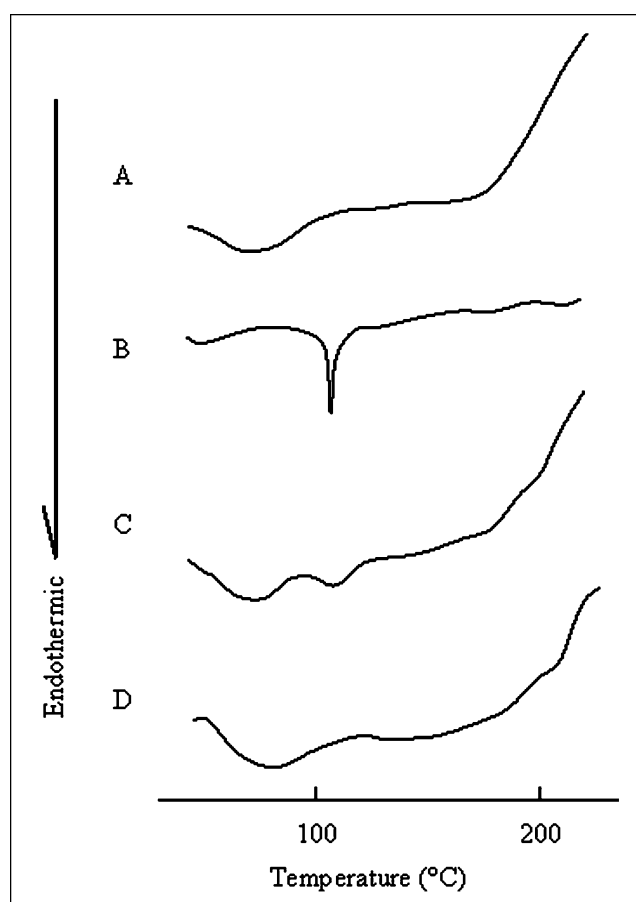


Fig. 4: DSC thermograms of FP-C-III systems. (A) C-III alone; (B) FP alone; (C) physical mixture of FP and C-III (1:3); (D) FP-C-III complex (1:3)

a rapid rate of dissolution and contained smaller amounts of LM chitosan was investigated more extensively. These results indicate that LM chitosans, especially C-III, can be useful for improving the dissolution rate of FP.

Figure 6 shows the dissolution profiles for FP from its kneaded mixtures containing LM chitosan in two different dissolution media, one of which is HCl (pH 1.2) and the other being phosphate buffer (pH 6.8) at 37°C . Both dissolution profiles show an instant supersaturation plateau. At pH 1.2 they dissolve rapidly and the dissolution curve then decreases with time by the effect of dilution of the dissolution medium.

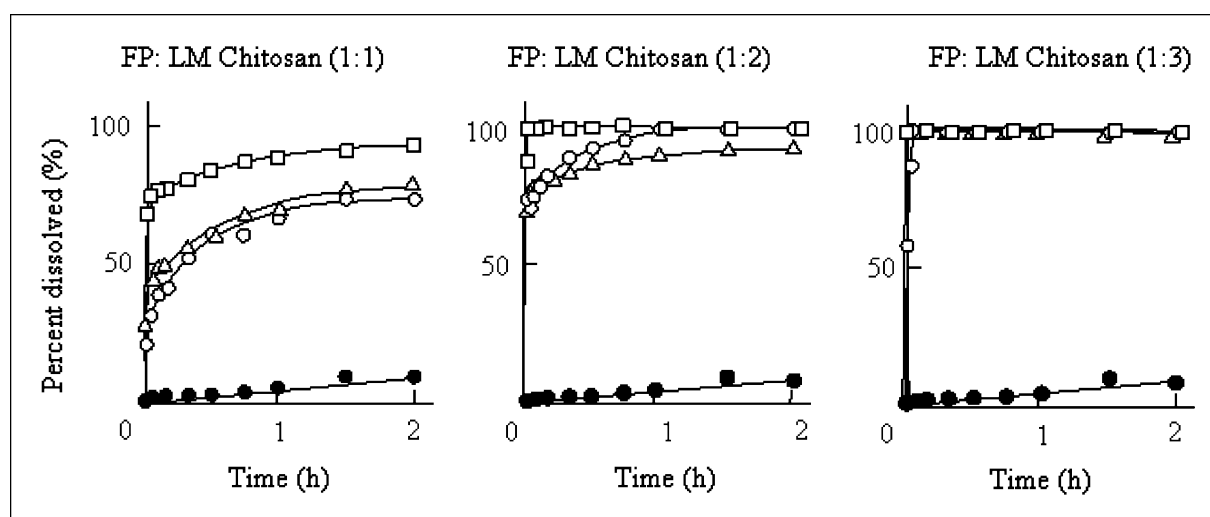


Fig. 5: Dissolution profiles for FP and its kneaded mixture with LM chitosans in water at 25°C . (●): FP alone (○) FP-C-I system; (△) FP-C-II system; (□) FP-C-III system. Each data point represents the average of 3 experiments

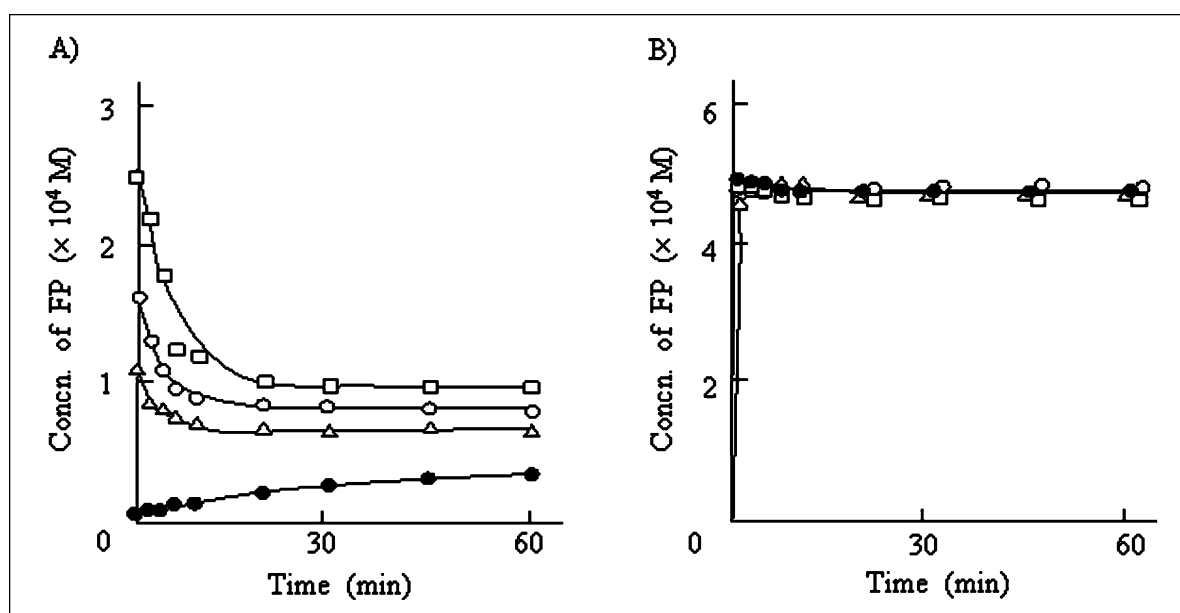


Fig. 6: Dissolution profiles of FP and its kneaded mixture with LM chitosans in JP XIV. No. 1 (A) or No. 2 (B) media at 37 °C. (●) FP alone; (○) FP-C-I system; (△) FP-C-II system; (□) FP-C-III system. Each data point represents the average of 3 experiments

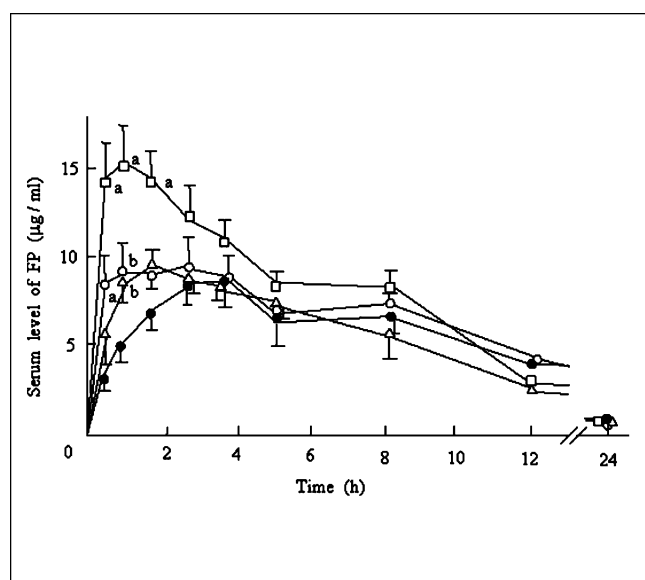


Fig. 7: Serum levels of FP following an oral administration of FP and its kneaded mixture with LM chitosans (equivalent to 5 mg/kg FP) to rats. (●) FP alone; (○) FP-C-I system; (△) FP-C-II system; (□) FP-C-III system. Values represent the mean \pm S.E. of 6 rats. ^a: $P < 0.01$ versus FP alone, ^b: $P < 0.05$ versus FP alone

2.4. In vivo absorption study

Figure 7 shows the plasma levels of FP following an oral administration of FP alone or its kneaded mixtures with C-I, C-II and C-III to male rats. The bioavailability parameters were calculated from the plasma level-time curves up to 24 h, post administration, and the results are summarized in Table 2. A significant difference in absorption rate constant (k_a) between FP and the kneaded mixture was observed. After the administration of FP, its concentration in plasma was negligible up to 0.5 h, and the T_{max} (time to reach maximum plasma concentration: C_{max}) value was 2–3 h. On the other hand, after administration of the kneaded mixtures, T_{max} values were 0.75, 1.08 and 0.5 h in the case of FP-C-I, FP-C-II and FP-C-III respectively. No significant

differences in C_{max} or the area under the plasma concentration-time curve (AUC) were observed between FP alone and its kneaded mixture with C-I and C-II. However, a significant difference in C_{max} and AUC was observed between FP alone and its kneaded mixture with C-III.

To elucidate the intestinal absorption enhancing mechanisms of LM chitosans, the intestinal absorption of FP alone and its kneaded mixture with C-III was compared using the *in situ* intestinal loop method. Changes in the amount of FP alone and C-III remaining in the loop are shown in Fig. 8. The remaining FP alone and C-III gradually decreased and no significant differences in the remaining were observed between FP alone and its kneaded mixture with C-III. These data indicate that the enhancement of absorption of the kneaded mixture after oral administration was due to the rapid dissolution into the GI fluid.

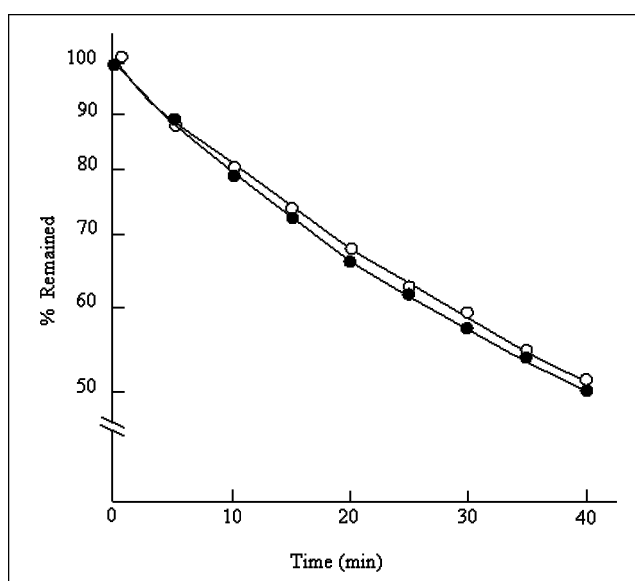


Fig. 8: Time course of the effect of C-III on FP absorption from rat small intestine. (●): FP alone, (○): FP-C-III (1:10)

Table 2: Pharmacokinetic parameters of FP and its kneaded mixtures with LM chitosans following oral administration to rats

Compd.	C _{max} (μg/ml)	T _{max} (h)	K _a (h ⁻¹)	AUC (h·μg/ml)	F (%)
FP alone	9.41 ± 1.53	2.08 ± 0.55	0.74 ± 0.83	111.64 ± 3.53	50.20 ± 2.53
FP-C-I	11.03 ± 2.54	0.75 ± 0.73	3.13 ± 1.23	115.15 ± 3.66	51.77 ± 3.24
FP-C-II	10.55 ± 1.69	1.08 ± 0.71	1.8 ± 1.11	101.39 ± 4.53	45.58 ± 4.53
FP-C-III	16.52 ± 2.61 ^a	0.50 ± 0.43 ^a	5.02 ± 1.03 ^a	129.79 ± 3.11 ^a	58.35 ± 2.51

Values represent the mean ± S.E. of 6 rats.

^a $p < 0.05$ in the kneaded mixture vs FP alone

2.5. Conclusion remarks

The present data suggests that LM chitosans, particularly C-III, interact with FP, and can be useful for improving dissolution and absorption rates of FP. In recent studies, many researchers reported that LM chitosans have a range of properties that are useful in biology and medicine (Harish Prashanth et al. 2005; Suzuki et al. 1987). A property of particular interest for this study is the antioxidant properties of chitosan. We measured the ability of LM chitosans preparation to protect human serum albumin from peroxy radicals and also showed that the administration of a chitosan supplement to human volunteers prevented albumin oxidation *in vivo* (Anraku et al. 2008; Anraku et al. 2009; Tomida et al. 2009). Based on the findings presented herein, it would be concluded that LM chitosans are potentially useful as DDS carriers and pharmaceutical additives such as solubilizer and antioxidant in pharmaceutical fields.

3. Experimental

3.1. Materials

Flurbiprofen (FP) was donated by the Sumitomo Pharmaceutical Co. Ltd. (Osaka, Japan). Low molecular weight chitosans (LM chitosans) were supplied by Kurita Water Industries Ltd. (Kanagawa, Japan). The physico-chemical properties of LM chitosans used in the studies are listed in Table 1. All other reagents and solvents were of analytical grade, and deionized double-distilled water was used throughout the study.

3.2. Solubility studies

Solubility measurements were carried out according to the method of Higuchi and Connors (1965). An excess amount of FP was added to an aqueous suspension containing various amounts of LM chitosans (0 ~ 10 w/v %), and the suspensions were then shaken at 25 °C. After reaching equilibrium (10 days), an aliquot of the suspension was centrifuged and filtered through a cellulose nitrate membrane filter (pore size 0.45 μm, Toyo Roshi Scientific Co. Ltd., Tokyo, Japan). A 2 ml aliquot of the sample solution was extracted with 10 ml of chloroform and the organic phase was analyzed spectrophotometrically at 247 nm.

3.3. Sample preparation

Mixtures of FP, kneaded with LM chitosan were prepared by the following method: FP and LM chitosan in weight ratios of 1:1, 1:2, and 1:3 were weighed and placed in a mortar, and the mixtures were then kneaded with 1.2 times their amount of water for 1 h. For example, in the case of the FP-LM chitosan (1:2) kneaded mixture, 1 g of FP and 2 g of LM chitosan were weighed and kneaded with 3.6 ml of water for 1 h. The resulting material was dried *in vacuo* at room temperature for 48 h. The fraction that passed a 100 mesh sieve was used in subsequent experiments. A physical mixture of FP with LM chitosan was prepared by mixing the powders (<100 mesh) in a mortar.

3.4. Apparatus

3.4.1. ¹³C NMR spectrometry

¹³C NMR spectra were recorded on a NMR spectrometer (JEOL JNM FX270) (Tokyo, Japan) at 20 ± 0.5 °C. FP and C-III were dissolved at a concentration of 5 mM in a mixture of CD₃OD and D₂O (1:1). Chemical shifts were calibrated indirectly through the use of external tetramethylsilane. The assignments for FP and chitosan were based on previously reported data from (Imai et al. 1991; Saitô et al. 1987).

3.4.2. Differential scanning calorimetry measurement

The differential scanning calorimeter (Thermo Flex Mode DSC10A; Rigaku Denki Co. Ltd., Tokyo, Japan) was operated at a scanning rate of 10 °C/min over a temperature range of 50 to 200 °C.

3.4.3. Powder X-ray diffraction studies

Powder X-ray diffraction patterns were obtained by scanning at 1°/min through a 2θ angle on a Rigaku Denki Geiger-flex-2012 diffractometer (Tokyo), using CuKα radiation; voltage, 40 kV; current, 30 mA; time constant, 1 s; scanning speed, 1°/min.

3.5. Dissolution studies

The dissolution of FP from samples was measured using the paddle method (JPXIV). The dissolution medium was 500 ml of 0.1 N HCl at pH 1.2 and a phosphate buffer solution at pH 6.8 at 37 °C with a stirring speed of 50 rpm. The amounts of sample powders used were 10 mg FP equivalent. Each sample powder (<100 mesh) was transferred directly into the dissolution medium and stirred with a stainless-steel paddle at 50 rpm. At appropriate intervals, 3 ml samples were removed from the flask, and filtered through a 0.45 μm membrane filter. The filtrate (1 ml) was extracted with 5 ml of chloroform separate out the LM chitosan. The drug concentration in the organic phase was determined spectrophotometrically at λ_{max} 247 nm. All studies were done in triplicate.

3.6. In vivo absorption studies

The experimental animals were 6 male rats, weighing 220 g, that were fasted for 24 h prior to administration of the drug. FP or a kneaded mixture with LM chitosan (equivalent to 5 mg/kg) was administered orally. At appropriate intervals, 0.5 ml of blood was withdrawn into heparinized syringes from the tail vein of rats, allowed to stand for 2 h then centrifuged at 3000 rpm for 20 min to give plasma samples.

The concentration of FP in the plasma samples was determined by HPLC. A 150 μl aliquot of serum was added to ibuprofen as an internal standard (30 μg/ml). FP was extracted with 6 ml of a benzene: ethyl acetate (1:1) solution, followed by shaking for 10 min and then centrifuged at 3000 rpm for 10 min., 10 μl of which was subjected to HPLC for the determination of FP. The HPLC conditions were as follows: pump and detector, Hitachi L-6200 type equipped with L-4000 UV monitor (Tokyo, Japan); column, Lichrosorb RP-18 (5 μm, 4 mm i.d. × 250 mm, Merck); mobile phase (0.5% acetic acid:methanol = 28:72, flow rate 1 ml/min; detection, 247 nm).

In situ intestinal perfusion was conducted in rats anesthetized with urethane (1.5 mg/kg, i.p.), using a 5 cm closed loop was prepared by ligating both ends of the selected segment after internally washing the small intestine (midgut) with saline about 20 cm below the duodeno-jejunal flexure. Solutions were prepared by adding FP (0.1 mg/mL) or a kneaded mixture with LM chitosan (10 mg/mL) to phosphate buffer (pH 6.4). After introducing 0.5 ml solution, the intestinal loop was returned to the abdominal cavity, and the abdomen was closed by sutures. For the determination of concentrations (FP or a kneaded mixture with LM chitosan) by above HPLC systems, 5 ml chloroform was added to 0.5 mL aliquots of perfusate samples.

3.7. Statistical analyses

Each value is expressed as the mean ± S.E. and statistical analysis was performed by the analysis of variance (ANOVA) with a oneway layout for comparisons with $P < 0.05$ as the minimum levels of significance.

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